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# Effects of aging and anatomic location on gene expression in human retina

***Hui Cai, Mark A. Fields, Risa Hoshino and Lucian V. Del Priore****†

*Department of Ophthalmology, Harkness Eye Institute, Columbia University, New York, NY, USA*

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***Correspondence:**
*Lucian V. Del Priore, Department of Ophthalmology, Harkness Eye Institute, Columbia University, 635 West 165th Street, New York, NY, USA.*
*e-mail: lvdelpriore@gmail.com*

†***Current address:***
*Lucian V. Del Priore, Department of Ophthalmology, Storm Eye Institute, Medical University of South Carolina, 167 Ashley Avenue, Charleston, SC 29425, USA.*

**Objective:** To determine the effects of age and topographic location on gene expression in human neural retina. **Methods:** Macular and peripheral neural retina RNA was isolated from human donor eyes for DNA microarray and quantitative RT-PCR analyses. **Results:** Total RNA integrity from human donors was preserved. Hierarchical clustering analysis demonstrates that the gene expression profiles of young, old, macula, and peripheral retina cluster into four distinct groups. Genes which are highly expressed in macular, peripheral, young, or old retina were identified, including inhibitors of Wnt Signaling Pathway (DKK1, FZD10, and SFRP2) which are preferably expressed in the periphery. **Conclusion:** The transcriptome of the human retina is affected by age and topographic location. Wnt pathway inhibitors in the periphery may maintain peripheral retinal cells in an undifferentiated state. Understanding the effects of age and topographic location on gene expression may lead to the development of new therapeutic interventions for age-related eye diseases.

**Keywords: human retina, macula, peripheral, aging, DNA microarray, gene expression, Wnt signaling pathway**

## INTRODUCTION

In the normal human eye the neural retina develops from mesoderm and forms a multilaminated structure with highly specialized functions for light detection and signal processing (Ye et al., 1999). During retinal aging, neuronal components of retina develop structural and functional changes that can adversely affect retinal function. Examples of age-dependent diseases of the retina include glaucoma and age-related macular degeneration (AMD), in which structural changes leading to visual loss develop in ganglion cells or outer retina, RPE, and choriocapillaris (Nag et al., 2006). The human macula, which is an anatomic region approximately 6 mm in diameter delineated by the optic nerve and the superior and inferior temporal vascular arcades, is adversely affected in AMD (Hornan et al., 2007). As a disease AMD is characterized by cellular changes in RPE, choriocapillaris, and outer retina and by structural changes in Bruch's membrane (Del Priore and Tezel, 1998; Spraul et al., 1999). Cellular changes that occur in AMD include atrophy of the RPE, choriocapillaris, and outer retina in non-exudative AMD as well as the development of choroidal or intraretinal neovascularization in exudative AMD (Chader, 2002). Ultimately some changes in cellular behavior may be initiated by or reflected in alterations in the gene expression profile of the cells (Radeke et al., 2007; Chen et al., 2008; Kurji et al., 2009; Stadler and Come, 2009). A systematic comparison of the gene expression profiles of young vs. older neural retina is thus important, as analysis of the retinal transcriptome may allow us to define a role for some genes in either initiating or responding to the cellular changes that occur in age-dependent diseases such as AMD. Topographic location may also affect gene expression profiling, since some diseases such as AMD affect the macula and periphery differently (van Soest et al., 2007).

To this end we have compared the gene expression profiles of young vs. old human neural retina, using both macular and peripheral neural retinal explants. In essence, macular and peripheral neural retinas were harvested from young and older human donor eyes and the retinal gene expression profiles were determined using the Affymetrix DNA microarray chip U133 plus 2. We were able to test the expression profile of 54,600 gene probes and determine genes whose expression level (mRNA) was altered by temporal (young vs. older) or spatial (macular vs. peripheral) factors. Knowledge of the function of genes with an altered expression profile may provide insight into the role of age-related changes in gene expression in the pathogenesis of human ocular disease.

## MATERIALS AND METHODS

### PREPARATION OF ADULT HUMAN RETINAL TISSUES

Twelve human donor eyes without recorded eye disease history from the National Disease Research Interchange (NDRI, Philadelphia, PA, USA) ranged in donor age from 18 to 79 years. Eyes were separated into a younger (18, 21, 32, 32, 35, and 43-year-old

**Table 1 | Human retina donor information.**

| Tissue ID | Death-to-enucleation time (h) | Enucleation to retina extraction time (h) | Age (year-old) | Gender and races | Cause of death |
|---|---|---|---|---|---|
| **FOR DNA MICROARRAY STUDY** | | | | | |
| Sample 1 | 7 | 11 | 18 | CM | Trauma |
| Sample 2 | 10 | 20 | 21 | CM | Breast cancer |
| Sample 3 | 3 | 26 | 32 | CM | Motor vehicle accident |
| Sample 4 | 2 | 29 | 32 | CF | Seizures |
| Sample 5 | 6 | 16 | 35 | CM | Cerebrovascular accident |
| Sample 6 | 10 | 18 | 43 | CF | Motor vehicle accident |
| Sample 7 | 8.5 | 23 | 72 | CM | Head trauma |
| Sample 8 | 2 | 21 | 74 | CM | Cardiac arrest |
| Sample 9 | 6.5 | 14 | 74 | CF | Breast cancer |
| Sample 10 | 10 | 10 | 74 | CM | Lung cancer |
| Sample 11 | 4 | 20 | 75 | CM | Anoxic encephalopathy |
| Sample 12 | 3.5 | 12 | 79 | CF | Respiratory failure |
| **FOR RT-PCR** | | | | | |
| Sample 13 | 7 | 10 | 34 | CM | Head trauma |
| Sample 14 | 13.5 | 10 | 38 | CM | Unknown |
| Sample 15 | 7 | 23 | 78 | BM | Cardiac arrest |
| Sample 16 | 5 | 10 | 81 | CM | colon cancer |

*Human retina donor information for microarray and real time qRT-PCR. All donor eyes were enucleated within 10 h of death and subsequently shipped to the lab within 32 h of death. All samples passed quality control using the hybridization signals from 3′, middle, and 5′ fragment of mRNA of housekeeping genes coded in the Affymetrix DNA chips (see **Figure 1**), suggesting that there is reasonable stability of RNA isolated from cadaver human donor eyes. C, Caucasian; B, black; M, male; F, female.*

**Table 2 | Primers used and the result of semi-quantitative RT-PCR compared to DNA microarray.**

| Gene Name | Gene Symbol | Oligo sequence | Fold changes | | Tissue compared |
|---|---|---|---|---|---|
| | | | Microarray | RT-PCR | |
| Protein tyrosine kinase-2 | PTK2 | Fwd-GCCTATTAAATGGATGGCTCCAG<br>Rev-AATTCGACCGATTACATCATTGTTCT | 2.9 | 2.3 | Young/old macula |
| Brain-derived neurotrophic factor | BDNF | Fwd-AAGATACATTTGTATGTTGTGAAGATGTTT<br>Rev-GCTTACTCTGACCAACGCC | 2.5 | 4.9 | Young/old macula |
| XIAP associated factor-1 | XAF1 | Fwd-CGAGCAGGGTTTCTTTATACTGG<br>Rev-TGTAGACTGCGTGGCACT | 2.3 | 2.5 | Young/old macula |
| Cadherin 8, type 2 | CDH8 | Fwd-CTACTGAAATTAGGAACCACAGTCAGAT<br>Rev-CTAACAGTTTGAATGACTTGGCCG | 2.2 | 3.0 | Young/old macula |
| Chloride intracellular channel 4 | CLIC4 | Fwd-CTGAATCACTTAAGAATTTCAGAATACCCT<br>Rev-ACCATGATTTATTGGGAGATGTTTATGTC | 3.0 | 2.5 | Old/young macula |
| Nuclear receptor co-repressor 2 | NCOR2 | Fwd-GGGCCACGTCATCTACGA<br>Rev-CTCCATCATGTCATAGGTGCG | 2.6 | 3.3 | Old/young macula |
| Dickkopf homolog 1 | DKK1 | Fwd-GGAATCCTGTACCCGGGC<br>Rev-CTGCAGGCGAGACAGATTTG | 6.6 | 2.4 | Periphery/macula (all ages) |
| Secreted frizzled-related protein 2 | SFRP2 | Fwd-GGAGATAACCTACATCAACCGAGATAC<br>Rev-GTCCCATGACCAGATAGGGC | 5.7 | 2.1 | Periphery/macula (all ages) |
| Frizzled homolog 10 | FZD10 | Fwd-CCGGCTTCGTGCTCATT<br>Rev-CAGCACAGAGAAGAGCCCGATA | 3.0 | 2.2 | Periphery/macula (all ages) |

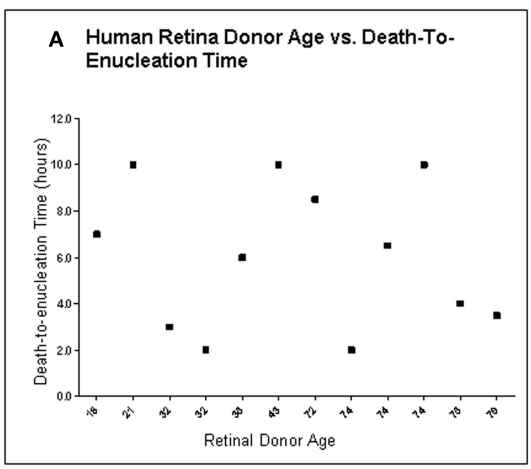
*Genes and corresponding oligonucleotide primers used for selective real time polymerase chain reaction (qRT-PCR). The last two columns show the ratio of mRNA expression levels from DNA microarray or qRT-PCR studies. Changes in expression are always in the same directions for qRT-PCR compared to microarray data, although the magnitude of the change can vary.*

cadaver donors) and older (72, 74, 74, 74, 75, and 79-year-old cadaver donors) age group. All donor eyes were enucleated within 10 h of death and processed in the lab within 32 h of death (**Table 1**). Since the study involved postmortem tissue without identification of individual patients it was exempt from Institutional Review Board (IRB) approval. Upon receipt in the laboratory, eyes were cleaned of extraocular tissue. The eyes were placed in carbon dioxide-free media (Gibco, Grand Island, NY, USA) and an incision was made through the sclera 3 mm posterior to the limbus and extended circumferentially. Four radial incisions were then made through the sclera and the sclera was peeled away. A full-thickness circumferential incision was made 1 mm posterior to the ora serrata; the anterior segment; and vitreous were removed and discarded. The posterior pole of each eyecup was inspected visually with direct and retroillumination under a dissecting microscope and globes were discarded if there was any evidence of subretinal blood, extensive drusen, or irregular pigmentation of the macular RPE. The choroid-Bruch's membrane-RPE complex was removed after trimming its attachment to the optic nerve using forceps, leaving the intact human retina as a flat mount. After rinsing three times with cold Dulbecco's Phosphate Buffered Saline (PBS) the macular retina was isolated from each eye using a 5-mm circular punch; a 5 mm punch of peripheral retina was then obtained by trephination of a circular region whose posterior border was at least 10 mm away from the macular punch. Cut tissues were rinsed again and stored at −80°C prior to isolating RNA. Twelve pairs of eyes from human donors were independently (not pooled samples) used for DNA microarray study; given the expense and availability of human tissue, similar small sample sizes have been used in the past to generate important data on gene expression in human tissue (Wistow et al., 2002; Chowers et al., 2003; Hollborn et al., 2005). Four additional retinal explants (donor age 34, 38, 78, and 81), independent of the samples used in the DNA microarray studies, were also harvested for confirmatory qRT-PCR (quantitative reverse transcriptase polymerase chain reaction) using independent samples (not pooled, **Table 1**).


**FIGURE 1 | Ratio of the 3′ and 5′ signal obtained from human house keeping gene GAPDH using microarray.** Study was performed to assess mRNA quality isolated from human donor eyes. Young samples and older samples are presented in order of donor age for both macular and peripheral samples. There is no correlation between donor age and signal ratio. The average of 3′/5′ signal ratio of all samples is 1.152 ± 0.07, which denotes good quality.

## ISOLATION OF TOTAL RNA

Human macula and peripheral retina were taken from a −80°C freezer and total RNA was isolated and purified using a Qiagen RNeasy Mini Kit according to manufacturer's instructions as described previously (Cai and Del Priore, 2006; Gong et al., 2008). Briefly, retinal tissue was disrupted and 600 µl of lysing buffer (RLT) was added to cells in a 1.5-ml microfuge tube. The cell lysate was loaded onto a QIAshredder spin column and spun for 2 min at 13,000 rpm. The homogenized lysate was then mixed



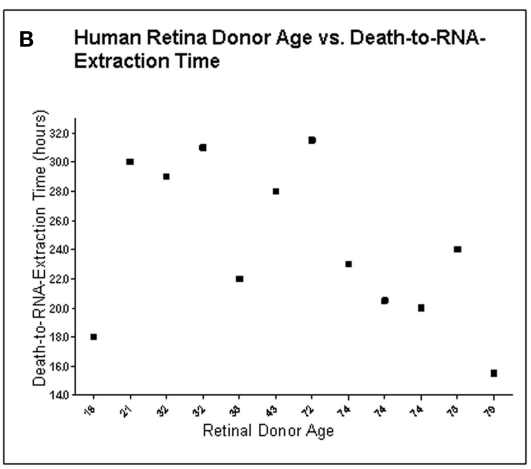




**FIGURE 2 | Human retina donor information. (A)** Human Retina Donor Age vs. Death-To-Enucleation Time **(B)**. Human Donor Age vs. Death-to-RNA-Extraction Time. Data showed that no bias was introduced by handling younger and older tissue differently, as there is no correlation between donor age and either death-to-enucleation or death-to-RNA isolation time.

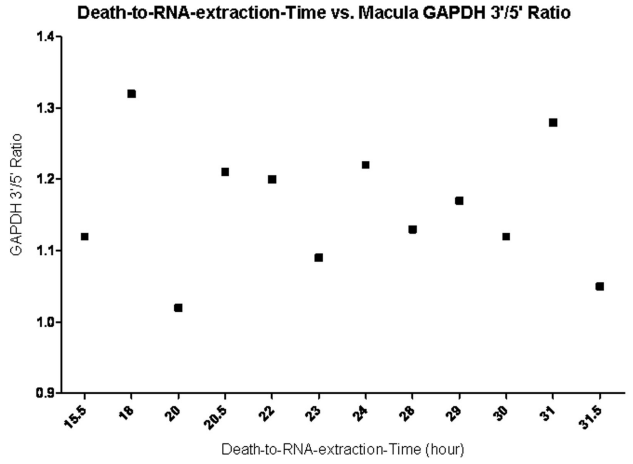



**FIGURE 3 | Death-to-RNA-Extraction time vs. GAPDH 3′/5′ ratio in macular tissue.** Data shows no correlation between enucleation time and the quality of RNA (the closer the ratio is to 1, the better the RNA quality) within the 31.5 h death-to-RNA-extraction time.

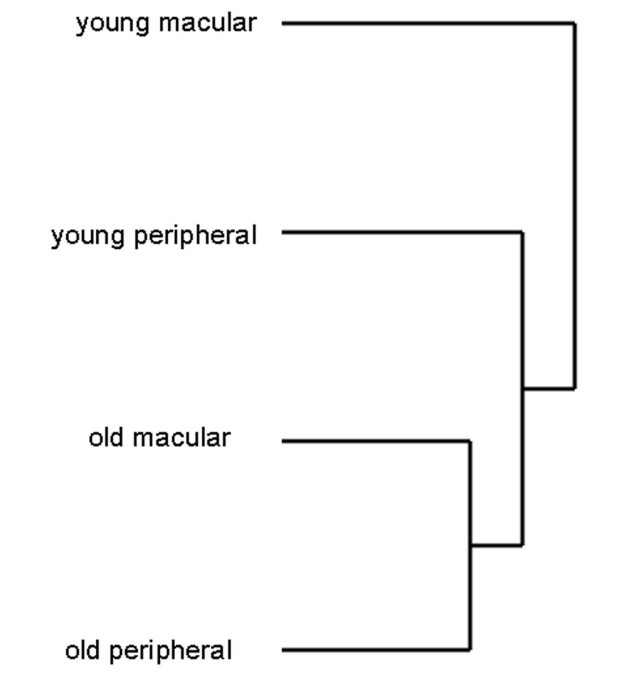



**FIGURE 4 | Hierarchical clustering analysis of retinal samples shows old and young retina (macular and peripheral) cluster separately, suggesting that aging changes the expression profile more than location (macula vs. periphery).** Young macular and peripheral samples were from different individuals for this analysis. Likewise older macular and peripheral samples were from different individual donors.

with 600 μl of 70% ethanol and applied to an RNeasy mini spin column and centrifuged for 15 s at 13,000 rpm. The specimen was then washed twice by adding 700 μl of Buffer RW1 and Buffer RPE, with subsequent spinning. Sixty microliters RNase-free water was used to elute total RNA from an RNeasy column. Approximately 8 μg of total RNA were extracted from macular and peripheral tissues (one punch each) of one pair of donor eyes. The quality of total RNA was assessed with the RNA denaturing agarose gel electrophoresis and microarray assay (see below).

### DNA MICROARRAY EXPERIMENTS

A T7-(dT)24 oligomer, superscript reverse transcriptase II and DNA Polymerase I (Gibco BRL) were used for first-strand and second-strand cDNA synthesis using 5 μg of total RNA as templates for each sample. Double-stranded cDNA was cleaned with Phase Lock Gels-Phenol/Chloroform extraction and ethanol precipitation. Biotin-labeled antisense cRNA was produced by an *in vitro* transcription reaction (ENZO BioArray High Yield RNA Transcript Labeling Kit) and incubated with fragmentation buffer (Tris-acetate, KOAc and MgOAc) at 94°C for 35 min. Target hybridization, washing, staining, and scanning probe arrays were done following an Affymetrix GeneChip Expression Analysis Manual. All human retinal samples are processed with individual microarray chips independently. The data then averaged/pooled for analysis and compared (MIAME accession # GSE32614).

### QUALITY CONTROLS, DEFINITIONS OF GENE PRESENCE OR ABSENCE AND STATISTICAL ANALYSIS

For assessing the quality of retinal RNA, 1% agarose gel with 0.22 M formaldehyde was used for RNA electrophoresis. One microgram of total RNA isolated from peripheral retinal samples was mixed with 2× loading buffer (Fisher Scientific) and run with 1× MOPS [3-(*N*-morpholino)propanesulfonic acid] buffer (Fisher Scientific). After ethidium bromide staining RNA bands were visualized with a UV transilluminator and 28S and 18S rRNA band patterns were analyzed.

For quality control the U133 plus 2 DNA microarray chips (Affymetrix, Santa Clara, CA, USA) used include housekeeping gene probes to measure the consistency of the hybridization signals from their 3′, middle, and 5′ fragment of these mRNA coding regions (Hubbell et al., 2002). Gene expression analyses, including global normalization, scaling, and Gene Ontology analysis were performed using the Affymetrix Expression Console (Ver. 1.1.) and GeneSifter Genetic Analysis system (Geospiza, Inc., Seattle, WA, USA). For the purpose of this study, gene expression was labeled as being differentially expressed if they were detected as present in the samples compared (i.e., young vs. old or macula vs. peripheral retina), had expression levels >50 in densitometry, and there was at least 2.0-fold difference in expression level that was statistically significant ($p < 0.05$ with Benjamini and Hochberg adjusted Student's *t*-test; Benjamini et al., 2001). We calculated a *z*-score to determine the relative gene expression changes (Doniger et al., 2003) and thereby identify biological processes, cellular components, and molecular group functions of those genes that warrant further study (Ashburner et al., 2000).

### REAL TIME QUANTITATIVE RT-PCR

Real time Quantitative RT-PCR (qRT-PCR) was performed on retinal samples harvested from different donors (ages 34, 38, 78, and 81 years) than those used to generate the microarray data (**Table 1**). The LightCycler system (Roche Diagnostics Corp.) was

**Table 3 | Genes highly expressed (up-regulated) in macula compared to peripheral retina.**

| Gene title | Gene Symbol | GO biological process term | Gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Peripherin | PRPH | Intermediate filament cytoskeleton organization | 8.3 | 2.45E−11 |
| POU class 4 homeobox 2 | POU4F2 | Negative regulation of transcription from RNA polymerase II promoter | 7.86 | 8.10E−12 |
| Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2 | SERPINE2 | Nervous system development | 6.87 | 8.73E−10 |
| Transmembrane protein 163 | TMEM163 | Integral to membrane | 6.72 | 1.02E−10 |
| Popeye domain containing 3 | POPDC3 | Integral to membrane | 6.54 | 4.22E−14 |
| AHNAK nucleoprotein 2 | AHNAK2 | Keratinization; cell differentiation | 6.33 | 1.86E−12 |
| POU class 4 homeobox 1 | POU4F1 | Nervous system development; axonogenesis; synaptogenesis | 6.03 | 1.10E−10 |
| Iroquois homeobox 2 | IRX2 | Regulation of transcription; transcription factor activity | 6.01 | 4.26E−13 |
| Sodium channel, voltage-gated, type I, beta | SCN1B | Ion transport | 5.81 | 7.36E−13 |
| Neurofilament, heavy polypeptide 200 kDa | NEFH | Nervous system development | 5.66 | 2.48E−11 |
| Early B-cell factor-1 | EBF1 | Regulation of transcription | 5.54 | 6.66E−12 |
| Annexin A2 | ANXA2 | Skeletal development | 5.51 | 3.93E−15 |
| Regulator of G-protein signaling 7 binding protein | RGS7BP | Negative regulation of signal transduction | 5.32 | 2.53E−06 |
| Fatty acid binding protein 3, muscle, and heart (mammary-derived growth inhibitor) | FABP3 | Negative regulation of cell proliferation | 5.25 | 4.97E−10 |
| Male sterility domain containing 1 | FAR2 | Lipid biosynthetic process; oxidation reduction | 5.15 | 2.07E−09 |
| Microtubule-associated protein 1A | MAP1A | Sensory perception of sound | 5.05 | 6.92E−10 |
| Peripheral myelin protein 2 | PMP2 | Establishment of localization; lipid binding | 4.93 | 3.04E−05 |
| Sushi-repeat-containing protein, X-linked | SRPX | Cell adhesion | 4.87 | 1.81E−08 |
| Iroquois homeobox 1 | IRX1 | Regulation of transcription | 4.76 | 4.48E−09 |
| Neurofilament, light polypeptide 68 kDa | NEFL | Neurofilament bundle assembly | 4.69 | 2.43E−11 |
| Sulfotransferase family 4A, member 1 | SULT4A1 | Lipid metabolic process | 4.65 | 2.13E−08 |
| Low density lipoprotein receptor (familial hypercholesterolemia) | LDLR | Lipid metabolic process | 4.58 | 7.77E−10 |
| Visinin-like 1 | VSNL1 | Neuronal calcium sensor | 4.38 | 6.37E−08 |
| RAB37, member RAS oncogene family | RAB37 | Protein transport | 4.32 | 8.19E−07 |
| 24-Dehydrocholesterol reductase | DHCR24 | Anti-apoptosis; response to oxidative stress; neuroprotection | 4.27 | 1.63E−10 |
| Complexin 1 | CPLX1 | Neurotransmitter transport; synaptic transmission | 4.23 | 1.38E−09 |
| ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 4 (Hu antigen D) | ELAVL4 | Cellular macromolecule metabolic process | 4.15 | 1.22E−08 |
| Sodium channel, voltage-gated, type IV, beta | SCN4B | Ion transport | 3.81 | 2.66E−12 |
| Cholinergic receptor, nicotinic, beta-3 | CHRNB3 | Signal transduction | 3.76 | 2.57E−08 |
| Adenylate cyclase 3 | ADCY3 | Intracellular signaling cascade; response to stimulus | 3.75 | 6.55E−11 |
| Multiple C2 domains, transmembrane 1 | MCTP1 | Calcium-mediated signal transduction | 3.72 | 5.52E−07 |
| RNA binding protein with multiple splicing 2 | RBPMS2 | Nucleotide binding | 3.72 | 1.98E−11 |
| Leucine rich repeat containing 8 family, member C | LRRC8C | Protein binding; integral to membrane | 3.68 | 1.28E−09 |
| Microtubule-associated monooxygenase, calponin, and LIM domain containing 2 | MICAL2 | Electron transport | 3.62 | 5.36E−10 |
| RNA binding protein with multiple splicing | RBPMS | RNA processing; nucleic acid binding | 3.58 | 2.10E−12 |
| Ras-like without CAAX 2 | RIT2 | Synaptic transmission | 3.5 | 4.66E−10 |
| GNAS complex locus | GNAS | Protein targeting; signal transduction | 3.46 | 7.02E−08 |
| Growth associated protein 43 | GAP43 | Nervous system development; cell differentiation | 3.35 | 1.44E−05 |
| Trophoblast glycoprotein | TPBG | Cell motility; cell adhesion | 3.32 | 5.90E−06 |

*(Continued)*

**Table 3 | Continued**

| Gene title | Gene Symbol | GO biological process term | Gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Brain expressed, associated with Nedd4 | BEAN | Protein binding; integral to membrane | 3.29 | 1.53E−10 |
| Sodium channel, voltage-gated, type I, alpha subunit | SCN1A | Ion transport | 3.28 | 3.83E−07 |
| Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase | MTHFD2 | One-carbon compound metabolic process | 3.28 | 2.33E−04 |
| Ectonucleoside triphosphate diphosphohydrolase 3 | ENTPD3 | Nucleoside diphosphate catabolic process | 3.22 | 4.16E−05 |
| Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2 | KCNN2 | Ion transport | 3.21 | 1.06E−09 |
| EPH receptor A4 | EPHA4 | Signal transduction; axon guidance | 3.1 | 7.37E−07 |
| ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 2 (Hu antigen B) | ELAVL2 | Regulation of transcription, DNA-dependent | 3.09 | 3.05E−08 |
| Eukaryotic translation initiation factor 5A2 | EIF5A2 | Translational initiation; polyamine homeostasis | 3.04 | 1.11E−10 |
| KISS1 receptor | KISS1R | Negative regulation of cell proliferation | 3.04 | 5.44E−08 |
| Leucine rich repeat and fibronectin type III domain containing 5 | LRFN5 | Intracellular membrane-bounded organelle | 3.02 | 1.63E−06 |
| Deleted in liver cancer 1 | DLC1 | Regulation of cell adhesion | 2.98 | 2.08E−11 |
| Neuritin 1 | NRN1 | Axonal regeneration; experimental diabetic neuropathy | 2.94 | 6.76E−11 |
| Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6 | SLC17A6 | Transport | 2.94 | 7.92E−11 |
| Synuclein, gamma (breast cancer-specific protein 1) | SNCG | Intracellular non-membrane-bounded organelle | 2.94 | 2.66E−08 |
| L1 cell adhesion molecule | L1CAM | Cell adhesion; nervous system development | 2.92 | 3.78E−11 |
| HIV-1 Tat interactive protein 2, 30 kDa | HTATIP2 | Regulation of apoptosis; regulation of angiogenesis | 2.9 | 7.43E−09 |
| Myocardial infarction associated transcript (non-protein coding) | MIAT | Associated with myocardial infarction | 2.9 | 1.88E−07 |
| Ankyrin 1, erythrocytic | ANK1 | Exocytosis; maintenance of epithelial cell polarity | 2.89 | 4.38E−08 |
| Synaptic vesicle glycoprotein 2C | SV2C | Neurotransmitter transport | 2.83 | 5.85E−10 |
| Eomesodermin homolog (*Xenopus laevis*) | EOMES | Anatomical structure morphogenesis; cell differentiation | 2.82 | 2.06E−09 |
| Heparan sulfate 6-*O*-sulfotransferase 3 | HS6ST3 | Transferase activity; integral to membrane | 2.73 | 3.05E−10 |
| Collagen, type IV, alpha 4 | COL4A4 | Long-term strengthening of neuromuscular junction | 2.71 | 3.25E−10 |
| Thy-1 cell surface antigen | THY-1 | Angiogenesis; retinal cone cell development; focal adhesion | 2.7 | 2.88E−10 |
| Cholinergic receptor, nicotinic, alpha 6 | CHRNA6 | Synaptic transmission | 2.68 | 7.26E−10 |
| Stearoyl-CoA desaturase (delta-9-desaturase) | SCD | Lipid metabolic process; iron ion binding | 2.66 | 2.77E−09 |
| Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase) | LSS | Lipid biosynthetic process | 2.63 | 6.13E−10 |
| Neurofilament, medium polypeptide 150 kDa | NEFM | Intermediate filament cytoskeleton organization and biogenesis | 2.63 | 2.85E−12 |
| Contactin 2 (axonal) | CNTN2 | Neuron migration; cell adhesion; integral to plasma membrane | 2.57 | 1.24E−08 |
| Vesicle-associated membrane protein 1 (synaptobrevin 1) | VAMP1 | Vesicle-mediated transport | 2.53 | 1.19E−12 |
| Hypothetical protein FLJ33996 | FLJ33996 | EST sequence; function known | 2.52 | 3.82E−09 |
| Lipin 1 | LPIN1 | Required for normal adipose tissue development | 2.43 | 3.08E−03 |

*(Continued)*

**Table 3 | Continued**

| Gene title | Gene Symbol | GO biological process term | Gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Thyroid hormone responsive (SPOT14 homolog, rat) | THRSP | Regulation of transcription; lipid metabolic process | 2.29 | 1.09E−09 |
| Calsyntenin 2 | CLSTN2 | Cell adhesion; calcium ion binding; postsynaptic membrane | 2.25 | 7.39E−08 |
| Stathmin-like 2 | STMN2 | Intracellular signaling cascade; neuron differentiation | 2.2 | 2.10E−11 |
| Early B-cell factor 3 | EBF3 | Regulation of transcription | 2.18 | 1.42E−05 |
| Glutaredoxin (thioltransferase) | GLRX | Electron transport | 2.16 | 4.73E−09 |
| SLIT-ROBO Rho GTPase activating protein 2 | SRGAP2 | GTPase activator activity | 2.15 | 3.63E−07 |
| Nicotinamide nucleotide adenylyltransferase 2 | NMNAT2 | NAD biosynthetic process; nucleotidyltransferase activity | 2.13 | 1.89E−07 |
| Protein phosphatase 2 (formerly 2A), regulatory subunit B, gamma isoform | PPP2R2C | Signal transduction | 2.09 | 3.15E−09 |
| Histone deacetylase 9 | HDAC9 | Regulation of transcription, DNA-dependent | 2.07 | 1.33E−07 |
| RAB15, member RAS oncogene family | RAB15 | Small GTPase mediated signal transduction; GTP binding | 2.04 | 5.61E−08 |
| Kinesin family member 5A | KIF5A | Microtubule-based movement; ATP-binding | 2.03 | 2.21E−07 |

*Genes highly expressed (up-regulated) in macular compared to peripheral retina (all ages). For changes in gene expression level we used a cutoff of >2.0-fold higher expression level in macular vs. peripheral retina ($p < 0.05$, Benjamini and Hochberg adjusted Student's t-test; expression level >50 on densitometry). These genes have a wide array of functions, including lipid metabolism, ion transport, neuronal differentiation and regulation of transcription, cell adhesion and motility, and differentiation.*

used for real time quantitative RT-PCR. An RNA Amplification Kit SYBR Green I (Roche Molecular Biochemicals, Mannheim, Germany) was used to synthesize the first-strand cDNA and subsequent amplification using gene specific primers (**Table 2**). The PCR reaction solution contains 0.5 μg of total RNA, 6 mM $MgCl_2$, and 0.5 μM of each primer. Other components in qRT-PCR master mix contain buffer, enzyme, SYBR green and dNTP. For reverse-transcription, reaction capillaries containing 20 μl RT-PCR reaction mix were incubated at 55˚C for 10 min, followed by incubation at 95˚C for 30 s. qRT-PCR was performed using an initial denaturation for 1 s at 95˚C, followed by 35 cycles of denaturation for 1 s at 95˚C, annealing for 10 s at 55˚C, and extension for 13 s at 72˚C in a programmable LightCycler. A melting curve analysis was performed by following the final cycle with incubation at 95˚C for 1 s, 65˚C for 10 s, followed by a temperature transition rate of 20˚C/s to reach 95˚C. Negative controls for the qRT-PCR analysis, which contained all reaction components except for RNA, were performed simultaneously to determine when the non-specific exponential amplification cycle number was reached.

## RESULTS

### SAMPLE QUALITY CONTROL ASSESSMENT

To determine the quality of the RNA isolated from human eyes, we determined the ratio of the 28S and 18S bands in the isolated RNA. The intensity ratio of 28S/18S was approximately 2.0: 1 without any significant smearing of the leading edges of either band (Data not shown). In addition a quality control analysis was performed using the hybridization signals from 3′, middle, and 5′ fragment of mRNA of endogenous housekeeping genes and exogenous "spiking" genes coded in the Affymetrix DNA chips (Hubbell et al., 2002; Archer and Guennel, 2006). All 24 samples passed the pre-established quality control criteria, which was detection of signal from each of the control genes (**Figure 1**). To exclude any potential bias due to differences in handling of young vs. older tissue, we demonstrated that there was no correlation between donor age and death-to-enucleation time (**Figure 2A**) or death-to-RNA-extraction time (**Figure 2B**). In addition, there was no correlation between the 3′/5′ ratio for the housekeeping gene GADPH and the death-to-RNA-extraction time (**Figure 3**).

### GLOBAL AND HIERARCHICAL CLUSTERING ANALYSIS

We detected the expression of approximately 26,700 gene probes out of 54,600 gene probes present on the Affymetrix Human Genome U133 plus 2 chip. There was no statistically significant difference in the total number of genes expressed between human young macula ($26686 \pm 319$), old macula ($26956 \pm 275$), young peripheral retina ($27122 \pm 108$), or old peripheral retina ($26533 \pm 490$; data not shown). There was also no statistically significant difference in the standard deviations of the number of genes expressed among these four groups ($F$-test, $p > 0.05$). Hierarchical clustering analysis of 24 samples showed that the transcriptome of the older macula and peripheral retina cluster together and young macula and peripheral retina cluster together as well (**Figure 4**), suggesting that there is a significant effect of aging on the gene expression profile of the human neural retina.

### GENE EXPRESSION ANALYSIS

There are 81 genes among approximately 26,700 gene probes that are expressed at higher levels (**Table 3**) in macula compared to peripheral retinal samples (combining all age groups) using the

**Table 4 | Genes highly expressed in peripheral compared to macular retina.**

| Gene title | Gene symbol | GO biological process term | Periphery vs. macula (all ages) gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Forkhead box G1 | FOXG1 | Regulation of transcription | 7.1 | 5.21E−14 |
| Dickkopf homolog 1 (*Xenopus laevis*) | DKK1 | Wnt receptor signaling pathway | 6.59 | 3.44E−05 |
| Secreted frizzled-related protein 2 | SFRP2 | Wnt receptor signaling pathway | 5.69 | 6.70E−08 |
| Hydroxysteroid (17-beta) dehydrogenase 2 | HSD17B2 | Lipid biosynthetic process | 5.08 | 9.67E−09 |
| Collagen, type II, alpha-1 (primary osteoarthritis, spondyloepiphyseal dysplasia, congenital) | COL2A1 | Phosphate transport | 4.54 | 4.42E−08 |
| Zic family member 1 (odd-paired homolog, *Drosophila*) | ZIC1 | Nervous system development | 3.68 | 2.40E−07 |
| Frizzled homolog 10 (*Drosophila*) | FZD10 | Wnt receptor signaling pathway | 2.96 | 2.12E−07 |
| Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein | ID3 | Negative regulation of transcription | 2.75 | 4.49E−04 |
| LIM homeobox 9 | LHX9 | Regulation of transcription, DNA-dependent | 2.69 | 1.21E−09 |
| Potassium inwardly rectifying channel, subfamily J, member 13 | KCNJ13 | Ion transport | 2.68 | 5.79E−05 |
| Histone cluster 2, H2aa3 | HIST2H2AA3 | Nucleosome assembly | 2.64 | 6.86E−04 |
| Zic family member 2 (odd-paired homolog, *Drosophila*) | ZIC2 | Multicellular organismal development | 2.53 | 1.70E−07 |
| Histone cluster 1, H2bb | HIST1H2BB | Nucleosome assembly | 2.52 | 3.43E−03 |
| Myoneurin | MYNN | Regulation of transcription, DNA-dependent | 2.48 | 1.51E−06 |
| Protein phosphatase 1, regulatory (inhibitor) subunit 3C | PPP1R3C | Carbohydrate metabolic process | 2.44 | 9.18E−06 |
| STEAP family member 4 | STEAP4 | Ion transport | 2.44 | 1.81E−06 |
| Histone cluster 1, H2bc | HIST1H2BC | Nucleosome assembly | 2.43 | 2.41E−03 |
| ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide | ATP1A2 | ATP biosynthetic process | 2.28 | 8.95E−06 |
| FXYD domain containing ion transport regulator 6 | FXYD6 | Ion transport | 2.28 | 6.45E−07 |
| Cysteine and glycine-rich protein 2 | CSRP2 | Multicellular organismal development | 2.26 | 6.46E−08 |
| Tigger transposable element derived 2 | TIGD2 | Cellular biopolymer biosynthetic process | 2.13 | 2.34E−05 |
| Nuclear receptor subfamily 4, group A, member 2 | NR4A2 | Nervous system development | 2.12 | 9.61E−03 |
| Rhodopsin | RHO | Rhodopsin mediated signaling pathway | 2.05 | 4.17E−05 |
| Histone cluster 1, H4b | HIST1H4B | Phosphoinositide-mediated signaling | 2.01 | 6.47E−03 |

*Genes highly expressed in peripheral compared to macular retina (all ages). For changes in gene expression level we used a cutoff of >2.0-fold higher expression level in peripheral vs. macular retina ($p < 0.05$, Benjamini and Hochberg adjusted Student's t-test; expression level >50 on densitometry). Note that DKK1, FZD10, and SFRP2 are expressed at higher levels in the peripheral retina than macular retina, suggesting that there is inhibition of the Wnt signaling pathway in the periphery compared to the human macula.*

definition described above. These genes have a wide array of functions, including lipid metabolism, ion transport, neuronal differentiation and regulation of transcription, cell adhesion and motility, and differentiation. There are 24 genes expressed at higher levels (**Table 4**) in the peripheral vs. macular retina (combining all age groups). These genes include those that are involved in the Wnt receptor signaling pathway, including DKK1 (Dickkopf homolog 1), FZD10 (frizzled homolog), and SFRP2 (secreted frizzled-related protein; **Table 4**; Robitaille et al., 2002; Kubo et al., 2003; Liu et al., 2007).

Aging alters the expression profile of numerous genes within the human macula. There are 85 genes that were expressed at higher levels (**Table 5**) in young macula compared to older macula. This includes genes with a diverse range of functions, including cell metabolism, cell regulation, development, and other cellular processes (**Figure 5A**). There are 55 genes that were expressed at higher levels (**Table 6**) in older compared to younger human macula. This includes genes with a wide role in cell proliferation, survival, and differentiation (**Figure 5B**).

There are 52 genes that were expressed at higher levels (**Table 7**) in younger peripheral vs. older peripheral retina. There are 34 genes that were expressed at higher levels (**Table 8**) in older vs. younger peripheral retina. The functions of these genes with

**Table 5 | Genes highly expressed in young compared to old macular retina.**

| Gene title | Gene symbol | GO biological process term | Young vs. older macula gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Chitinase 3-like 1 (cartilage glycoprotein-39) | CHI3L1 | Carbohydrate metabolic process | 9.26 | 1.80E−04 |
| Peripheral myelin protein 2 | PMP2 | Transport; lipid binding | 5.31 | 6.88E−04 |
| Interferon-induced protein with tetratricopeptide repeats 3 | IFIT3 | Receptor binding | 3.86 | 1.96E−02 |
| Fatty acid binding protein 5 (psoriasis-associated) | FABP5 | Lipid metabolic process | 3.82 | 5.56E−04 |
| Autocrine motility factor receptor | AMFR | Ubiquitin cycle; signal transduction | 3.76 | 3.47E−08 |
| Chemokine (C-X-C motif) ligand 2 | CXCL2 | Inflammatory response; G-protein-coupled signaling pathway | 3.43 | 4.36E−03 |
| Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1) | SERPINE1 | TGF-beta signaling pathway; regulation of angiogenesis | 3.43 | 9.28E−03 |
| Cytoplasm; intracellular part | IFI44L | Interferon-induced protein 44-like | 3.27 | 8.04E−04 |
| Matrix Gla protein | MGP | Cartilage condensation; cell differentiation m ion | 3.19 | 4.62E−03 |
| Defensin, beta 119 | DEFB119 | Defense response | 3.05 | 2.68E−02 |
| Immune response | GBP1 | Guanylate binding protein 3 | 3.04 | 7.91E−04 |
| metabolic process | MICAL2 | Microtubule-associated monooxygenase, calponin, and LIM domain containing 2 | 2.99 | 7.62E−03 |
| Nucleoside diphosphate catabolic process | ENTPD3 | Ectonucleoside triphosphate diphosphohydrolase 3 | 2.98 | 4.88E−03 |
| Regulation of transcription, DNA-dependent | BACH2 | BTB and CNC homology 1, basic leucine zipper transcription factor 2 | 2.96 | 5.92E−05 |
| Angiogenesis | ELK3 | ELK3, ETS-domain protein (SRF accessory protein 2) | 2.96 | 2.81E−02 |
| Cell-substrate junction assembly | ITGA6 | Integrin, alpha 6 | 2.95 | 4.23E−02 |
| Protein binding | SNCG | Synuclein, gamma (breast cancer-specific protein 1) | 2.94 | 2.45E−06 |
| Regulation of ARF protein signal transduction | PSD3 | Pleckstrin and Sec7 domain containing 3 | 2.93 | 3.15E−03 |
| Regulation of transcription, DNA-dependent | STAT1 | Signal transducer and activator of transcription 1, 91 kDa | 2.93 | 2.52E−04 |
| Positive regulation of cell proliferation | TIMP1 | TIMP metallopeptidase inhibitor 1 | 2.93 | 2.31E−03 |
| ATP biosynthetic process | ATP2B3 | ATPase, Ca++ transporting, plasma membrane 3 | 2.92 | 5.38E−03 |
| Ubiquitin-dependent protein catabolic process | PSMB9 | Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2) | 2.92 | 5.17E−03 |
| Extracellular region | CCDC80 | Coiled-coil domain containing 80 | 2.91 | 7.56E−04 |
| Multicellular organismal development | ELAVL3 | ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 3 (Hu antigen C) | 2.91 | 7.25E−04 |
| Regulation of transcription, DNA-dependent | NHLH2 | Nescient helix-loop-helix 2 | 2.91 | 3.08E−03 |
| Glutamate decarboxylation to succinate | GAD1 | Glutamate decarboxylase 1 (brain, 67 kDa) | 2.9 | 1.15E−02 |
| Suckling behavior | POU4F1 | POU class 4 homeobox 1 | 2.9 | 2.46E−03 |
| Protein tyrosine kinase-2 | PTK2 | Neuron migration; cell motility; integrin-mediated signaling pathway | 2.9 | 7.78E−12 |
| Protein modification process | PCMTD2 | Protein-l-isoaspartate (d-aspartate) *O*-methyltransferase domain containing 2 | 2.89 | 6.55E−03 |
| Ion transport | SCN2B | Sodium channel, voltage-gated, type II, beta | 2.89 | 9.94E−03 |
| Ion transport | KCNQ2 | Potassium voltage-gated channel, KQT-like subfamily, member 2 | 2.88 | 1.21E−03 |
| Structural molecule activity | MAP1A | Microtubule-associated protein 1A | 2.88 | 5.96E−06 |
| Multicellular organismal development | EMP1 | Epithelial membrane protein 1 | 2.8 | 1.14E−02 |

*(Continued)*

**Table 5 | Continued**

| Gene title | Gene symbol | GO biological process term | Young vs. older macula gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Vitronectin | VTN | Inflammatory response pathway; cell adhesion | 2.7 | 1.42E−03 |
| Cell adhesion | TPBG | Trophoblast glycoprotein | 2.64 | 6.54E−03 |
| CART prepropeptide | CARTPT | neuropeptide signaling pathway; transmission of nerve impulse | 2.62 | 7.06E−03 |
| One-carbon compound metabolic process | MTHFD2 | Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase | 2.62 | 2.81E−02 |
| Golgi associated PDZ and coiled-coil motif-containing | GOPC | ER to Golgi vesicle-mediated transport; Golgi to plasma membrane transport | 2.6 | 9.99E−04 |
| Chromosome 8 open reading frame 4 | C8orf4 | Apoptosis | 2.57 | 1.79E−02 |
| Tumor necrosis factor receptor superfamily, member 12A | TNFRSF12A | Angiogenesis; apoptosis; cell motility | 2.53 | 4.51E−04 |
| Brain-derived neurotrophic factor | BDNF | Positive regulation of neuron differentiation; anti-retinal programmed cell death | 2.51 | 2.19E−03 |
| Protein phosphatase 2 (formerly 2A), regulatory subunit B, gamma isoform | PPP2R2C | Signal transduction; protein phosphatase type 2A regulator activity | 2.51 | 3.18E−07 |
| Solute carrier family 1 (neuronal/epithelial glutamate transport) | SLC1A1 | Transport; dicarboxylic acid transport; synaptic transmission | 2.49 | 2.79E−03 |
| Mitogen-activated protein kinase kinase kinase 14 | MAP3K14 | Protein amino acid phosphorylation | 2.47 | 5.84E−06 |
| Signal transduction | GNG4 | Guanine nucleotide binding protein (G-protein), gamma 4 | 2.46 | 5.07E−03 |
| Paraneoplastic antigen MA2 | PNMA2 | Transport | 2.46 | 2.38E−04 |
| Thyrotropin-releasing hormone | TRH | Cell-cell signaling; hormone-mediated signaling | 2.44 | 1.08E−03 |
| mRNA catabolic process | HSPA1B | Heat shock 70 kDa protein 1B | 2.4 | 2.95E−02 |
| Selenocysteine incorporation | DIO2 | Deiodinase, iodothyronine, type II | 2.39 | 9.55E−03 |
| Cell surface receptor linked signal transduction | IFITM1 | Interferon-induced transmembrane protein 1 (9–27) | 2.39 | 7.94E−04 |
| Interleukin 8 | IL8 | Angiogenesis; cell motility; chemotaxis | 2.39 | 1.33E−02 |
| Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) | MX1 | Induction of apoptosis; defense response | 2.38 | 1.17E−02 |
| Regulation of cell growth | TMEM97 | Transmembrane protein 97 | 2.38 | 1.09E−03 |
| Cas-Br-M (murine) ecotropic retroviral transforming sequence | CBL | Cell surface receptor linked signal transduction | 2.37 | 1.28E−02 |
| Type I interferon biosynthetic process | IRF9 | Interferon regulatory factor 9 | 2.36 | 1.71E−04 |
| Dihydropyrimidinase-like 2 | DPYSL2 | Nucleobase, nucleoside, nucleotide, and nucleic acid metabolism | 2.35 | 1.17E−03 |
| p21 (CDKN1A)-activated kinase 6 | PAK6 | Protein amino acid phosphorylation | 2.34 | 4.12E−05 |
| Zinc finger protein 441 | ZNF441 | Transcription; regulation of transcription, DNA-dependent | 2.32 | 5.24E−04 |
| Ubiquitin specific peptidase 31 | USP31 | Ubiquitin-dependent protein catabolism; ubiquitin cycle | 2.29 | 3.81E−03 |
| Transmembrane 4 L six family member 1 | TM4SF1 | Integral to plasma membrane | 2.28 | 5.72E−03 |
| Protein binding | DTX3L | Deltex 3-like (*Drosophila*) | 2.26 | 1.66E−02 |
| Response to stress | HSPA1A | Heat shock 70 kDa protein 1A | 2.26 | 5.88E−03 |
| Kruppel-like factor 7 (ubiquitous) | KLF7 | Transcription; regulation of transcription from RNA polymerase II promoter | 2.26 | 2.42E−04 |
| XIAP associated factor-1 | XAF1 | Apoptosis; negative regulation of cell cycle | 2.26 | 3.94E−05 |
| Pleckstrin homology domain containing, family G (with RhoGef domain) | PLEKHG4 | Cell death; regulation of Rho protein signal transduction | 2.24 | 4.53E−04 |

*(Continued)*

**Table 5 | Continued**

| Gene title | Gene symbol | GO biological process term | Young vs. older macula gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Complement component 1, r subcomponent | C1R | Proteolysis; complement activation, classical pathway | 2.21 | 5.17E−03 |
| Cadherin 8, type 2 | CDH8 | Cell adhesion; homophilic cell adhesion; cell adhesion | 2.2 | 2.40E−03 |
| Regulation of cell growth | GAP43 | Growth associated protein 43 | 2.19 | 1.32E−03 |
| Regulation of translational initiation | HSPB1 | Heat shock 27 kDa protein 1 | 2.18 | 4.51E−02 |
| Keratinization | AHNAK2 | AHNAK nucleoprotein 2 | 2.17 | 1.04E−03 |
| Regulation of cell growth | CD44 | CD44 molecule (Indian blood group) | 2.16 | 4.18E−02 |
| Regulation of neurotransmitter levels | GABRA2 | Gamma-aminobutyric acid (GABA) A receptor, alpha 2 | 2.14 | 5.48E−03 |
| Regulation of cell growth | SOCS3 | Suppressor of cytokine signaling 3 | 2.12 | 1.09E−04 |
| Protein import into nucleus, docking | XPO1 | Exportin 1 (CRM1 homolog, yeast) | 2.1 | 9.11E−03 |
| Proteolysis | PAPPA | Pregnancy-associated plasma protein A, pappalysin 1 | 2.09 | 7.73E−04 |
| Apoptosis | PRUNE2 | Prune homolog 2 (*Drosophila*) | 2.09 | 6.46E−04 |
| Protein binding | TRIM9 | Tripartite motif-containing 9 | 2.09 | 9.77E−03 |
| Intrinsic to membrane | MYADM | Myeloid-associated differentiation marker | 2.08 | 6.84E−06 |
| FOS-like antigen 2 | FOSL2 | Regulation of transcription from RNA polymerase II promoter; cell death | 2.07 | 3.65E−02 |
| Protein amino acid O-linked glycosylation | LDLR | Low density lipoprotein receptor (familial hypercholesterolemia) | 2.07 | 1.68E−04 |
| Cell adhesion | FAT3 | FAT tumor suppressor homolog 3 (*Drosophila*) | 2.04 | 1.44E−02 |
| Carbohydrate metabolic process | FUT9 | Fucosyltransferase 9 (alpha (1,3) fucosyltransferase) | 2.04 | 3.84E−03 |
| Regulation of transcription, DNA-dependent | IRX1 | Iroquois homeobox 1 | 2.02 | 1.78E−02 |
| Histidine catabolic process | MOXD1 | Monooxygenase, DBH-like 1 | 2.01 | 4.51E−04 |
| Response to unfolded protein | HSPH1 | Heat shock 105/110 kDa protein 1 | 2.00 | 1.73E−02 |

*For changes in gene expression level we used a cutoff of >2.0-fold higher expression level in young vs. older macula ($p < 0.05$, Benjamini and Hochberg adjusted Student's t-test; expression level >50 on densitometry). Note that the expression level of genes known to be important for retinal survival/protection such as X-linked inhibitor of apoptosis (XAF1), Cadherin (CDH8), PTK2 protein tyrosine kinase (PTK2), and brain-derived neurotrophic factor (BDNF) decrease in the aging retina.*

high *z*-scores (Doniger et al., 2003) are grouped with ontology (**Figures 5C,D**).

### SELECTIVE QUANTITATIVE RT-PCR

We then performed semi-quantitative RT-PCR on several selected genes that were altered in the microarray data that had played a role in retinal survival, cellular apoptosis, or were involved in the Wnt pathway. These genes code for the X-linked inhibitor of apoptosis (XAF1, Renwick et al., 2006), cadherin 8 (CDH8, Chen and Ma, 2007), protein tyrosine kinase-2 (PTK2, Finnemann, 2003), brain-derived neurotrophic factor (BDNF, Wilson et al., 2007), nuclear receptor co-repressor 2 (NCOR2, Tsai et al., 2004; Jepsen et al., 2007), chloride intracellular channel 4 (CLIC4, Chen et al., 2004), DKK1, FZD10, and SFRP2 (Robitaille et al., 2002; Kubo et al., 2003; Liu et al., 2007). In all cases, the changes in expression level detected with the microarray data are in the same direction (i.e., up or down) as the changes in expression level detected by qRT-PCR. There is reasonable agreement between the relative expression level of each of these genes when comparing younger vs. older samples as detected by microarray data and qRT-PCR (**Table 2**).

## DISCUSSION

We have used the Affymetrix DNA microarray chip U133 plus 2 to study the gene expression profile of the human retina as a function of age and topographic location (macula vs. peripheral retina). We were able to confirm the microarray findings with qRT-PCR of selected genes. There is some variation in the exact relative expression level between qRT-PCR and microarray data, but in every case, the relative expression levels using the microarray data and qRT-PCR were always in the same direction (**Table 2**).

We were able to detect the presence of approximately 26,700 out of 54,600 gene probes present on the Affymetrix Human Genome U133 plus 2 chip in all four groups; namely, young macula, young periphery, older macula, and older periphery. The number of gene probes that we detected in human retina is about 10,000 more than those reported in RNA extracted from human retinal ganglion cells (Kim et al., 2006). This was probably due to the fact that our samples contained multiple retinal cell types. *A priori* we reasoned that aging of the macula and/or periphery might increase either the number of genes expressed throughout the retina or the variation in the number of genes expressed in older peripheral vs.

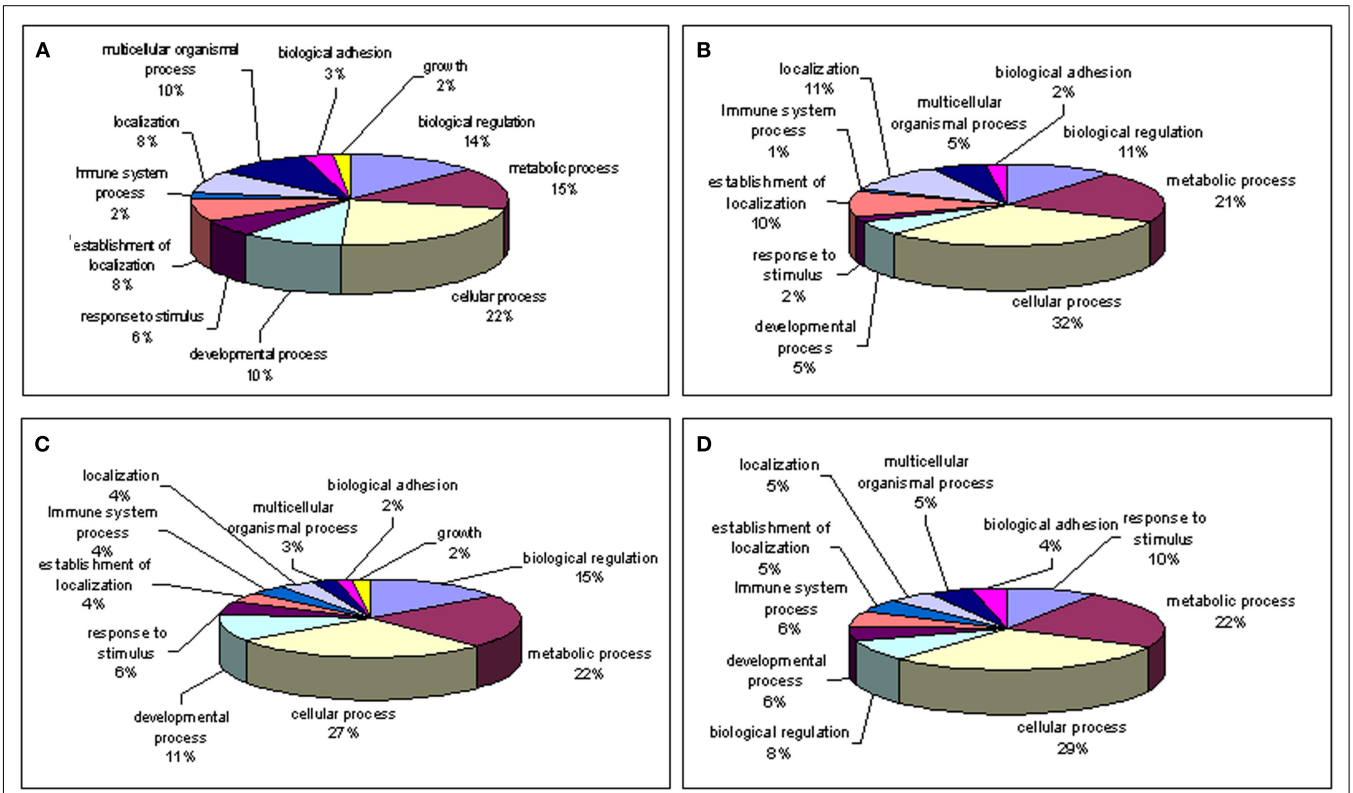



**FIGURE 5 | Gene ontology of biological processes (high *z*-score) involved with genes. (A)** Up-regulated; **(B)** down-regulated in young macula compared with older macula; **(C)** up-regulated; **(D)** down-regulated in young compared with older peripheral retina. Note that genes involved with "growth" are only shown in young macula and peripheral retina but missing in older macula and periphery.

macular samples; however, there was no significant difference in the average number or standard deviation of the number of genes expressed in young vs. older macular or peripheral samples (data not shown).

Hierarchical clustering analysis is a statistical technique used to sort heterogeneous samples into several distinct groups that contain genes with similar expression patterns (Eisen et al., 1998; Krajewski and Bocianowski, 2002). Clustering analysis suggests that aging changes the expression profile more than the location of retina (macular vs. peripheral; **Figure 4**). To circumvent the possibility that the macula from a donor is simply clustering with the periphery from the same donor, this analysis was repeated with a smaller subset of eyes so that young macula and young peripheral samples were obtained from unrelated individuals, as were young and old peripheral samples. This did not alter the clustering pattern seen in **Figure 4** (data not shown).

Previous authors have also sought to determine the retinal gene expression profile as a function of age in both macular and peripheral retina using smaller sample sizes (Yoshida et al., 2002; Hornan et al., 2007; Ben-Shlomo et al., 2008). Yoshida et al. developed gene expression profiles of young and elderly human retinas using microarray slides containing 2400 human genes that were primarily neuronal. More than 50% hybridized to the retinal cDNA targets. Northern blot analysis and qRT-PCR results confirmed the changes in expression in 8 of 10 genes examined, including an increase in IFN-responsive transcription factor subunit (ISGF3G), creatine kinase B (CKB), and pancreatic amylase (AMY2A), and a decrease in TGF-beta receptor interacting protein 1 (TRIP1), LPS-induced TNF-alpha factor (PIG7), alpha-1 (E)-catenin (CTNNA1), ubiquitin hydrolase (USP9X), GABA receptor beta-3 subunit (GABRB3), and alpha-1 Type VII collagen (COL7A1). Hornan et al. compared the expression profile of cone-rich macular vs. rod rich peripheral retina using 2–4 mm retinal punches from human retina, and demonstrated that macula transcripts were enriched for nuclear pore complex interacting protein (NPIP) and eukaryotic translation initiation factor 2 alpha kinase (GCN2), with these protein products being detected in cone outer segments. Ben-Shlomo et al. examined the gene expression profile over the first 20 weeks of life in rat retina dissected during the first 20 weeks of life at 2 different time points and identified 603 differentially expressed genes, which were grouped into six clusters based on changes in expression levels during the first 20 weeks of life. A bioinformatic analysis of these clusters revealed sets of genes encoding proteins with functions relevant to retinal maturation, such as potassium, sodium, calcium, and chloride channels, synaptic vesicle transport, and axonogenesis. Schippert et al. (2009) compared the expression profile of wild type and Egr-1 knockout mice, which have longer eyes and a more myopic refractive error compared to their wild-types. Changes in expression were confirmed in four genes by RT-PCR, including nuclear prelamin A recognition factor (Narf), oxoglutarate dehydrogenase (Ogdh), selenium binding protein 1 (Selenbp1),

**Table 6 | Genes highly expressed in old compared to young macula.**

| Gene title | Gene symbol | GO biological process term | Older vs. young macula gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Dickkopf homolog 1 (*Xenopus laevis*) | DKK1 | Negative regulation of Wnt receptor signaling pathway | 5.98 | 2.55E−10 |
| G-protein-coupled receptor 177 | GPR177 | Positive regulation of I-kappaB kinase/NF-kappaB cascade | 3.43 | 3.58E−03 |
| Triadin | TRDN | Muscle contraction | 3.35 | 2.05E−03 |
| Synapse defective 1, Rho GTPase, homolog 2 (*C. elegans*) | SYDE2 | Signal transduction | 3.24 | 2.24E−03 |
| Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin) | ENPP2 | Phosphate metabolic process | 3.21 | 6.16E−05 |
| Prolactin | PRL | Prostaglandin synthesis regulation; cell surface receptor linked signal transduction | 3.18 | 3.93E−03 |
| Solute carrier family 6 (neurotransmitter transporter, taurine), member 6 | SLC6A6 | Beta-alanine transport | 3.11 | 9.26E−03 |
| Potassium inwardly rectifying channel, subfamily J, member 13 | KCNJ13 | Potassium ion transport | 3.09 | 3.37E−06 |
| Epididymal sperm binding protein 1 | ELSPBP1 | Single fertilization | 3.04 | 6.30E−03 |
| Aquaporin 4 | AQP4 | Nervous system development | 2.98 | 1.42E−05 |
| Chloride intracellular channel 4 | CLIC4 | Negative regulation of cell migration; transport; chloride transport | 2.98 | 8.59E−03 |
| Rho GTPase activating protein 29 | ARHGAP29 | Rho protein signal transduction | 2.95 | 1.98E−04 |
| ATP-binding cassette, subfamily G (WHITE), member 1 | ABCG1 | Lipid transport | 2.78 | 1.50E−04 |
| Cerebellar degeneration-related protein 2, 62 kDa | CDR2 | Regulation of translation | 2.74 | 4.51E−03 |
| Cell division cycle 42 (GTP binding protein, 25 kDa) | CDC42 | Nuclear migration | 2.66 | 9.55E−04 |
| Cell division cycle associated 7 | CDCA7 | Transcription; regulation of transcription | 2.66 | 1.05E−04 |
| ADAM metallopeptidase with thrombospondin type 1 motif | ADAMTS5 | Proteolysis; protein amino acid prenylation; proteolysis | 2.63 | 1.89E−04 |
| Cyclin-dependent kinase inhibitor 3 | CDKN3 | Cell cycle; cell cycle arrest; negative regulation of cell proliferation | 2.6 | 6.04E−05 |
| Nuclear receptor co-repressor 2 | NCOR2 | Negative regulation of transcription, DNA-dependent | 2.58 | 2.05E−05 |
| Palmdelphin | PALMD | Regulation of cell shape | 2.57 | 2.38E−03 |
| 5-Nucleotidase, ecto (CD73) | NT5E | DNA metabolic process | 2.51 | 4.99E−03 |
| Sarcospan (Kras oncogene-associated gene) | SSPN | Muscle contraction; cell adhesion | 2.51 | 4.11E−05 |
| Ras responsive element binding protein 1 | RREB1 | Ras protein signal transduction | 2.45 | 3.41E−06 |
| Zinc finger and BTB domain containing 1 | ZBTB1 | Transcription; regulation of transcription, DNA-dependent | 2.41 | 8.92E−03 |
| Lin-7 homolog C (*C. elegans*) | LIN7C | Neurotransmitter secretion | 2.4 | 6.13E−05 |
| Zic family member 1 (odd-paired homolog, *Drosophila*) | ZIC1 | Brain development | 2.39 | 5.63E−04 |
| Tetraspanin 2 | TSPAN2 | Cell motility; cell adhesion; cell proliferation | 2.36 | 8.05E−03 |
| Hydroxysteroid (17-beta) dehydrogenase 2 | HSD17B2 | Steroid biosynthetic process | 2.35 | 5.87E−03 |
| ATP-binding cassette, subfamily A (ABC1), member 4 | ABCA4 | Transport; visual perception; phototransduction, visible light | 2.34 | 5.58E−03 |
| Metallothionein 1F | MT1F | Copper ion binding | 2.34 | 6.45E−03 |

*(Continued)*

**Table 6 | Continued**

| Gene title | Gene symbol | GO biological process term | Older vs. young macula gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| ATPase, H+ transporting, lysosomal 56/58 kDa | ATP6V1B1 | Ossification; ion transport; sensory perception of sound | 2.33 | 8.70E−03 |
| Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein | ID3 | Negative regulation of transcription from RNA polymerase II promoter | 2.33 | 3.51E−02 |
| UDP-GlcNAc:betaGal beta-1,3-*N*-acetylglucosaminyltransferase 7 | B3GNT7 | Protein amino acid glycosylation | 2.32 | 2.01E−03 |
| Solute carrier family 26 (sulfate transporter), member 2 | SLC26A2 | Inorganic anion transport | 2.32 | 3.10E−08 |
| Coiled-coil and C2 domain containing 1A | CC2D1A | Positive regulation of I-kappaB kinase/NF-kappaB cascade | 2.31 | 1.88E−04 |
| Growth arrest-specific 7 | GAS7 | Cell cycle arrest | 2.31 | 1.57E−04 |
| Leucine rich repeat containing 57 | LRRC57 | Protein binding | 2.31 | 2.57E−04 |
| Cholecystokinin | CCK | Neuron migration; axonogenesis; neuropeptide hormone activity | 2.3 | 1.49E−04 |
| Collagen, type II, alpha-1 | COL2A1 | Visual perception | 2.3 | 5.61E−04 |
| Cytochrome P450, family 26, subfamily B, polypeptide 1 | CYP26B1 | Cell fate determination; retinoic acid receptor signaling pathway | 2.29 | 2.97E−07 |
| Calsequestrin 1 (fast-twitch, skeletal muscle) | CASQ1 | Calcium ion binding | 2.28 | 5.97E−03 |
| Protein phosphatase 1, regulatory (inhibitor) subunit 3C | PPP1R3C | Carbohydrate metabolic process | 2.27 | 2.43E−04 |
| Chloride intracellular channel 5 | CLIC5 | Ion transport | 2.26 | 2.25E−05 |
| Matrix-remodeling associated 7 | MXRA7 | Integral to membrane | 2.26 | 6.69E−04 |
| Kelch-like 14 (*Drosophila*) | KLHL14 | Protein binding | 2.25 | 6.75E−06 |
| Phosphodiesterase 1A, calmodulin-dependent | PDE1A | Signal transduction; signal transduction | 2.25 | 1.75E−03 |
| Carboxylesterase 1 (monocyte/macrophage serine esterase 1) | CES1 | Metabolic process | 2.24 | 3.28E−03 |
| RAB23, member RAS oncogene family | RAB23 | Signal transduction; nervous system development | 2.24 | 1.64E−02 |
| Myeloid cell nuclear differentiation antigen | MNDA | Regulation of macromolecule metabolic process | 2.22 | 5.92E−03 |
| Family with sequence similarity 108, member B1 | FAM108B1 | Hydrolase activity | 2.21 | 6.61E−03 |
| Zinc finger, DBF-type containing 2 | ZDBF2 | Nucleic acid binding | 2.21 | 3.18E−05 |
| Tigger transposable element derived 4 | TIGD4 | Regulation of transcription | 2.19 | 1.30E−03 |
| 2-Oxoglutarate and iron-dependent oxygenase domain containing 1 | OGFOD1 | Protein metabolic process | 2.17 | 4.45E−03 |
| Heterogeneous nuclear ribonucleoprotein F | HNRNPF | RNA splicing, via transesterification reactions | 2.15 | 9.09E−03 |
| Enolase 3 (beta, muscle) | ENO3 | Cellular macromolecule catabolic process | 2.07 | 4.54E−03 |

*Genes highly expressed in old compared to young macula retina. For changes in gene expression level we used a cutoff of >2.0-fold higher expression level in old vs. young macula ($p < 0.05$, Benjamini and Hochberg adjusted Student's t-test; expression level >50 on densitometry). Note that aging increased the expression of genes related to aging and apoptosis, such as genes coding for the nuclear receptor co-repressor 2 (NCOR2) and chloride intracellular channel 4 (CLIC4).*

and Pcdhb9. Glenn et al. (2009) showed that glycation of the basement membrane causes a significant reduction in cathepsin-D activity in ARPE-19 ($p < 0.05$) and an increase in lipofuscin accumulation ($p < 0.01$). Chen et al. (2008) compared the transcriptional profiles of the RPE/choroid from young and old mice. There were 315 genes differentially expressed with age; most of these genes were related to immune responses and inflammatory activity. There was increased gene expression and protein levels of leukocyte attracting signal, chemokine ligand 2 (Ccl2) in aged RPE/choroid. These studies cover a wide range of conditions, including using different array chips, and comparing young vs. old, and macula vs. peripheral, in several species, including humans. Despite these differences, our data (**Tables 3**, **4**, and **6**) is consistent with prior published studies showing up-regulation of HNRPF

**Table 7 | Genes highly expressed in young compared to old peripheral retina.**

| Gene title | Gene symbol | GO biological process term | Young vs. older periphery gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Heat shock 70 kDa protein 6 (HSP70B′) | HSPA6 | Response to stress; response to unfolded protein | 9.2 | 1.54E−02 |
| Histone cluster 1, H2bc | HIST1H2BC | nucleosome assembly | 5.16 | 8.79E−03 |
| Autocrine motility factor receptor | AMFR | Ubiquitin cycle; ER-associated protein catabolic process | 4.69 | 1.39E−08 |
| Chitinase 3-like 1 (cartilage glycoprotein-39) | CHI3L1 | Carbohydrate metabolic process | 3.81 | 8.35E−03 |
| Heat shock 70 kDa protein 1B | HSPA1B | Anti-apoptosis; response to stress | 3.78 | 3.17E−03 |
| Phorbol-12-myristate-13-acetate-induced protein 1 | PMAIP1 | Release of cytochrome c from mitochondria | 3.69 | 2.91E−03 |
| Histone cluster 2, H2aa3/histone cluster 2, H2aa4 | HIST2H2AA3 | Nucleosome assembly | 3.61 | 8.23E−03 |
| CDC14 cell division cycle 14 homolog B (*S. cerevisiae*) | CDC14B | Protein amino acid dephosphorylation | 3.42 | 2.23E−03 |
| Ciliary rootlet coiled-coil, rootletin-like 1 | CROCCL1 | Structural component of the ciliary rootlet | 3.09 | 9.20E−05 |
| CART prepropeptide | CARTPT | Activation of MAPKK activity | 2.97 | 9.13E−03 |
| Calcium channel, voltage-dependent, L type, alpha-1D subunit | CACNA1D | Ion transport | 2.96 | 2.47E−04 |
| Heat shock 70 kDa protein 1A | HSPA1A | Anti-apoptosis; response to stress | 2.96 | 8.55E−03 |
| Integrin, alpha 6 | ITGA6 | Cell-substrate junction assembly | 2.95 | 9.69E−03 |
| Glutathione-*S*-transferase theta 1 | GSTT1 | Glutathione metabolic process | 2.94 | 1.97E−03 |
| Histone cluster 1, H2bh | HIST1H2BH | Nucleosome assembly | 2.94 | 1.68E−03 |
| GUF1 GTPase homolog (*S. cerevisiae*) | GUF1 | Nucleotide binding | 2.92 | 5.45E−05 |
| Syntaxin binding protein 6 (amisyn) | STXBP6 | Vesicle-mediated transport | 2.92 | 9.20E−04 |
| Growth arrest and DNA-damage-inducible, gamma | GADD45G | Activation of MAPKKK activity; response to stress; cell differentiation | 2.84 | 8.31E−03 |
| SLIT-ROBO Rho GTPase activating protein 1 | SRGAP1 | Signal transduction | 2.73 | 5.44E−04 |
| Spectrin, beta, non-erythrocytic 1 | SPTBN1 | Barbed-end actin filament capping | 2.7 | 7.17E−05 |
| Vitronectin | VTN | Inflammatory response pathway; histidine biosynthetic process | 2.6 | 8.44E−03 |
| Enhancer of polycomb homolog 1 (*Drosophila*) | EPC1 | Regulation of cell growth; transcription | 2.54 | 2.59E−03 |
| Choroideremia (Rab escort protein 1) | CHM | Blood vessel development; visual perception | 2.49 | 1.49E−03 |
| Monooxygenase, DBH-like 1 | MOXD1 | Histidine catabolism; catecholamine metabolism | 2.44 | 6.69E−04 |
| Sideroflexin 4 | SFXN4 | Protein biosynthesis; transport; ion transport | 2.42 | 5.52E−03 |
| ATG9 autophagy related 9 homolog B | ATG9B | Autophagic vacuole formation | 2.37 | 7.15E−03 |
| Mdm2, transformed 3T3 cell double minute 2 | MDM2 | Negative regulation of transcription | 2.37 | 9.13E−03 |
| Fatty acid binding protein 5 (psoriasis-associated) | FABP5 | Lipid metabolic process | 2.36 | 3.22E−03 |
| Ring finger protein 103 | RNF103 | Central nervous system development | 2.35 | 8.83E−03 |
| Solute carrier family 20 (phosphate transporter), member 1 | SLC20A1 | Phosphate metabolic process | 2.35 | 1.42E−03 |
| Mortality factor 4 like 2 | MORF4L2 | Regulation of cell growth | 2.31 | 3.55E−02 |
| Secreted frizzled-related protein 2 | SFRP2 | Somitogenesis, Wnt signaling pathway | 2.31 | 3.75E−03 |
| Zinc finger and BTB domain containing 24 | ZBTB24 | Cellular biopolymer biosynthetic process | 2.31 | 8.73E−03 |
| Zinc finger protein 664 | ZNF664 | Regulation of transcription, DNA-dependent | 2.29 | 5.14E−03 |
| RNA binding motif protein 4 | RBM4 | mRNA processing; RNA splicing | 2.28 | 6.20E−03 |
| Protein tyrosine phosphatase, receptor type, G | PTPRG | Protein amino acid dephosphorylation | 2.27 | 2.51E−02 |
| Prostaglandin reductase 1 | PTGR1 | Leukotriene metabolic process | 2.26 | 4.13E−03 |

*(Continued)*

**Table 7 | Continued**

| Gene title | Gene symbol | GO biological process term | Young vs. older periphery gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Quaking homolog, KH domain RNA binding (mouse) | QKI | Multicellular organismal development | 2.25 | 6.86E−05 |
| Neuronal PAS domain protein 4 | NPAS4 | Regulation of transcription, DNA-dependent | 2.19 | 3.13E−03 |
| Hypothetical protein KIAA1434 | RP5-1022P6.2 | Carbohydrate metabolic process | 2.17 | 2.33E−06 |
| Growth associated protein 43 | GAP43 | Regulation of cell growth | 2.16 | 9.22E−03 |
| Musashi homolog 2 (*Drosophila*) | MSI2 | Nucleotide binding | 2.16 | 9.72E−03 |
| Phosphodiesterase 4D interacting protein (myomegalin) | PDE4DIP | Cytoskeleton organization | 2.16 | 4.60E−06 |
| SVOP-like | SVOPL | Establishment of localization | 2.16 | 3.48E−04 |
| Ubiquitin-like modifier activating enzyme 6 | UBA6 | Protein modification process | 2.13 | 5.69E−03 |
| Serine/threonine kinase receptor associated protein | STRAP | mRNA processing | 2.12 | 2.17E−03 |
| Rap guanine nucleotide exchange factor (GEF) 2 | RAPGEF2 | MAPKKK cascade | 2.1 | 9.72E−10 |
| Exportin 1 (CRM1 homolog, yeast) | XPO1 | Protein import into nucleus, docking | 2.09 | 1.60E−03 |
| Ring finger protein 12 | RNF12 | Regulation of transcription, DNA-dependent | 2.02 | 1.88E−03 |
| Splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor) | SFRS1 | Nuclear mRNA splicing, via spliceosome | 2.02 | 2.68E−03 |
| Solute carrier family 6 (neurotransmitter transporter, GABA), member 13 | SLC6A13 | Neurotransmitter transport | 2.01 | 1.77E−03 |
| 5-Nucleotidase, ecto (CD73) | NT5E | DNA metabolic process | 2.00 | 9.69E−03 |

*For changes in gene expression level we used a cutoff of >2.0-fold higher expression level in young vs. older peripheral retina ($p < 0.05$, Benjamini and Hochberg adjusted Student's t-test; expression level >50 on densitometry).*

(heterogeneous nuclear ribonucleoprotein F) and ENO3 (Muscle specific enolase) in older retina; (Yoshida et al., 2002) higher expression levels of RHO (rhodopsin) in periphery; and higher expression levels of HDAC9 (histone deacetylase 9) and SRGAP2 (Rho GTPase activating protein 2) in the macula (Hornan et al., 2007).

It is interesting to note that there are only 24 genes expressed at higher levels in the periphery vs. macular retina and 3 of these genes (namely, DKK1, FZD10, and SFRP2) encode for protein products that inhibit the Wnt receptor signaling pathway (**Table 4**). There are three major types of inhibitors of this pathway in *Xenopus* that have human homologs, including the secreted frizzled-related proteins (sFRPs; Melkonyan et al., 1997), Wnt-inhibitory factor-1 (WIF-1; Hsieh et al., 1999), and Dickkopf (DKK), which also includes four known human proteins DKK1–4 (Krupnik et al., 1999). Wnt ligands belong to a highly conserved family of oncogenes expressed in species ranging from the fruit fly to man (McMahon and Moon, 1989; Busse and Seguin, 1993; Magee, 1995). Wnt signaling controls many events during embryogenesis and exerts significant regulation of cell morphology, proliferation, motility, and cell fate (Parr and McMahon, 1994; Siegfried and Perrimon, 1994; Turnbull et al., 1995). Inappropriate activation of the Wnt signaling pathway has been observed in several human cancers (Spink et al., 2000). Inhibition of the Wnt pathway is correlated with preventing cells from moving into a regenerative state, and Wnt signaling is important in transdifferentiation of ciliary margin stem cells into neural retina at the ciliary marginal zone (Robitaille et al., 2002; Kubo et al., 2003; Liu et al., 2007). Addition of Wnt3a to cultures of ciliary margin cells increased the number of proliferating cells and allowed the cells to maintain their multilineage potential (Inoue et al., 2006; Liu et al., 2007). Wnt signaling may provide a therapeutic strategy for *in vitro* expansion or *in vivo* activation of adult retinal stem cells (Inoue et al., 2006; Liu et al., 2007). Our observation that DKK1, FZD10, and SFRP2 are expressed at higher levels in the peripheral retina than those in macular retina (**Table 4**) suggests that there is inhibition of the Wnt signaling pathway in the periphery compared to the macular human retina. A potential strategy for cell replacement in retinal disorders, including retinitis pigmentosa (Pruett, 1983; Smith et al., 2009), is to activate this pathway in the peripheral retina and ciliary marginal zone.

We were able to detect genes whose expression levels change with aging of the human neural retina, and many of these genes appear to be related to cell growth, proliferation, and survival. For instance, aging decreases the expression level of genes known to be important for retinal survival/protection such as X-linked inhibitor of apoptosis (XAF1), Cadherin (CDH8), PTK2 protein tyrosine kinase (PTK2), and BDNF. Aging increases the expression of genes related to aging and apoptosis, such as genes coding for the nuclear receptor co-repressor 2 (NCOR2) and chloride intracellular channel 4 (CLIC4; **Tables 2**, **5**, and **6**). These changes may explain the increasing susceptibility of the human retina to

**Table 8 | Genes highly expressed in old compared to young peripheral retina.**

| Gene title | Gene symbol | GO biological process term | Older vs. young periphery gene expression fold-change | *p*-Value |
|---|---|---|---|---|
| Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin) | ENPP2 | Phosphate metabolic process | 3.45 | 9.41E−05 |
| Cholecystokinin | CCK | Neuron migration | 3.42 | 1.38E−04 |
| Sarcolipin | SLN | Regulation of calcium ion transport | 3.13 | 1.98E−04 |
| Ribosomal protein S26 | RPS26 | Negative regulation of RNA splicing | 2.99 | 1.42E−07 |
| 2-Oxoglutarate and iron-dependent oxygenase domain containing 1 | OGFOD1 | Protein metabolic process | 2.98 | 9.68E−03 |
| Laminin, alpha 3 | LAMA3 | Cell adhesion | 2.96 | 2.20E−04 |
| Zinc finger protein 43 | ZNF43 | Cellular biopolymer biosynthetic process | 2.95 | 1.17E−03 |
| Metallothionein 1 M | MT1M | Copper ion binding | 2.94 | 6.54E−03 |
| Ribosomal protein L31 | RPL31 | Biopolymer biosynthetic process | 2.92 | 3.08E−04 |
| Leucine rich repeat containing 57 | LRRC57 | Protein binding | 2.9 | 9.45E−04 |
| Peptidase inhibitor 15 | PI15 | Endopeptidase inhibitor activity | 2.9 | 6.76E−05 |
| Matrix-remodeling associated 7 | MXRA7 | Integral to membrane | 2.71 | 1.41E−03 |
| Ras suppressor protein 1 | RSU1 | Ras protein signal transduction | 2.64 | 2.15E−03 |
| Tudor domain containing 6 | TDRD6 | Germ cell development | 2.53 | 2.82E−10 |
| Zinc finger and BTB domain containing 1 | ZBTB1 | Transcription, DNA-dependent | 2.52 | 2.04E−03 |
| Cell division cycle 42 (GTP binding protein, 25 kDa) | CDC42 | Small GTPase mediated signal transduction | 2.49 | 1.07E−03 |
| REV1 homolog (*S. cerevisiae*) | REV1 | DNA repair; response to UV; response to DNA damage | 2.46 | 2.00E−04 |
| Ras responsive element binding protein 1 | RREB1 | Ras protein signal transduction | 2.41 | 5.15E−08 |
| Growth arrest-specific 7 | GAS7 | Cell cycle arrest | 2.39 | 1.12E−04 |
| Myo-inositol 1-phosphate synthase A1 | ISYNA1 | Inositol biosynthetic process | 2.36 | 9.41E−03 |
| Thymidylate synthetase | TYMS | Nucleobase, nucleoside, nucleotide, and nucleic acid metabolic process | 2.36 | 9.37E−04 |
| Rho GTPase activating protein 29 | ARHGAP29 | Rho protein signal transduction | 2.31 | 3.53E−04 |
| Zinc finger, DBF-type containing 2 | ZDBF2 | Nucleic acid binding | 2.17 | 1.68E−04 |
| Pyruvate dehydrogenase phosphatase isoenzyme 2 | PDP2 | Protein amino acid dephosphorylation | 2.11 | 6.79E−06 |
| Chromosome 18 open reading frame 1 | C18orf1 | Intrinsic to membrane | 2.08 | 4.66E−05 |
| Synovial sarcoma translocation, chromosome 18 | SS18 | Intracellular membrane-bounded organelle | 2.05 | 3.43E−03 |
| Solute carrier family 6 (neurotransmitter transporter, taurine), member 6 | SLC6A6 | Beta-alanine transport | 2.03 | 3.64E−02 |
| Crystallin, mu | CRYM | Visual perception | 2.02 | 6.02E−04 |
| Fatty acid binding protein 4, adipocyte | FABP4 | Cytokine production | 2.02 | 7.35E−04 |
| Heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A) | HNRNPU | Nuclear mRNA splicing, via spliceosome | 2.02 | 4.53E−03 |
| Doublecortex; lissencephaly, X-linked (doublecortin) | DCX | Neuron migration | 2.01 | 1.67E−03 |
| Enhancer of zeste homolog 1 (*Drosophila*) | EZH1 | Cellular biopolymer biosynthetic process | 2.01 | 2.44E−04 |
| Metallothionein 1G | MT1G | Copper ion binding | 2.01 | 2.07E−03 |
| NLR family, pyrin domain containing 2 | NLRP2 | Apoptosis, positive regulation of caspase activity | 2.00 | 3.32E−03 |

*For changes in gene expression level we used a cutoff of >2.0-fold higher expression level in old vs. young peripheral retina ($p < 0.05$, Benjamini and Hochberg adjusted Student's t-test; expression level >50 on densitometry).*

some diseases as patient age increases, such as AMD and glaucoma. Retinal aging is also associated with changes in expression of genes involved in the complement cascade; the relationship of altered expression of these genes to the development of age-related diseases such as AMD remains to be elucidated. Any individual change or combination of changes may be responsible

for altering retinal gene expression (Cai et al., 2006; Han et al., 2007).

Our gene ontology analysis (Wu et al., 2006; Noel et al., 2007; Grigoryev et al., 2008) reveals genes whose expression levels change during retina aging are involved in cellular metabolism, regulation of the cell cycle, cell adhesion, and other biological pathways (**Figure 5**). Interestingly, up-regulated genes involved with cell growth were detected only within younger macula and peripheral retina (**Figures 5A,C**) but not in older macula and peripheral retina (**Figures 5B,D**).

We recognize that an intrinsic limitation of using human tissue is the potential RNA degradation that can occur between death and RNA isolation; in our view this limitation is counterbalanced by the fact that the value of data obtained from human retina cannot be replaced by other means. Several facts suggest that retinal RNA can be relatively stable between death and RNA isolation within the time frame we used. First, Malik et al. (2003) conducted an RNA stability study on neural retina and RPE and concluded that the RNA from neural retina was stable up to 48 h after death. In the current study we used a cutoff of 32 h for the death-to-RNA harvesting time, which is within the period of time that retinal RNA is stable. Although proteins and RNA degrade by different mechanisms, there is also tremendous stability of the retinal proteome after harvesting, as there was no significant time-dependent change in intensity for >95% of retinal proteins examined up to 48 h postmortem (Ethen et al., 2006). Second, we measured the relative intensity of the 28S and 18S RNA bands and demonstrated that there was no significant RNA degradation at the time of RNA isolation (data not shown). Third, we demonstrated that there is no significant degradation of the signal from housekeeping genes, as revealed by the stability of the hybridization signals from 3′, middle, and 5′ fragment of mRNA of housekeeping genes coded in the Affymetrix DNA chips (**Figures 1** and **3**). Fourth, we did not introduce any bias by handling younger and older tissue differently, as there is no correlation between donor age and either death-to-enucleation or death-to-RNA isolation time (**Figure 2**).

There are other potential limitations to our study. First, it is likely that there is significant patient-to-patient variation in gene expression profiling, particularly since our samples may include patients with normal eyes as well as patients with age-related disease or dysfunction. Second, we harvested full-thickness human retina for this analysis. Thus, mixed retinal cell types were present within our full-thickness retinal punches. Additional studies are necessary and planned to determine which cell(s) contribute to the changes in gene expression seen here. Third, there is incomplete correlation between the transcriptome and proteomics of many tissues (Hack, 2004; Baginsky et al., 2005; Cox et al., 2007; Fagan et al., 2007; Hesketh et al., 2007; Dihal et al., 2008). Additional studies are necessary to determine the effects of aging and topographic location on retinal proteomics. Fourth, our study does not consider the effects of aging and/or topographic location on post-translational protein modification; these effects have been shown to be significant in other ocular tissues, including lens (Takemoto and Gopalakrishnan, 1994). As with any other gene expression studies we cannot discern whether the gene expression changes that we observe are primary or secondary. Despite these limitations we have obtained important information on changes in the gene expression that occur in aging human retina. Additional studies are required to determine the role of specific alterations in the transcriptome in the pathogenesis of age-related ocular diseases such as AMD.

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